

# Study Supports QF-PCR Test as Proxy for FISH, Karyotyping in Prenatal Aneuploidy Detection

February 17, 2011

## Study Supports QF-PCR Test as Proxy for FISH, Karyotyping in Prenatal Aneuploidy Detection

By Ben Butkus

**A molecular test** based on quantitative fluorescent PCR is faster, less complex, and at least as accurate as more established methods such as karyotyping and fluorescence *in situ* hybridization for prenatal detection of common aneuploidies, according to a study published this month by Canadian researchers.

The study adds to a growing body of evidence that QF-PCR testing has the potential to replace the current gold standard of karyotyping for prenatal aneuploidy detection in Canada and, eventually, the US, according to experts.

However, the test, called the Aneufast QF-PCR kit and manufactured by Spanish molecular diagnostics outfit Molgentix, first needs 510(k) clearance in the US, which depends on whether the Applied Biosystems genetic analyzer on which Aneufast is performed is also approved for diagnostic use, Ray O' Connor, CEO of Molgentix parent company and Aneufast distributor Genomed, told *PCR Insider* this week.

"I don't know how long it will take them to get FDA approval, but the day they get [it], we will start selling our test in the US," O'Connor said.

Aneufast has the CE IVD Mark and is already commonly used for prenatal aneuploidy detection in Europe and Canada, among other regions of the world, O'Connor said, although he declined to disclose specific sales figures.

The test is based on the relative quantitation of polymorphic markers on each of several chromosomes of interest using fluorescently labeled primers to amplify the markers followed by fragment analysis on a Life Technologies Applied Biosystems genetic analyzer.

More specifically, the kit contains six multiplex marker sets of short tandem repeats that can be used to amplify selected microsatellites as well as the amelogenin and SRY loci. This combination of markers allows the detection of aneuploidies involving chromosomes X, Y, 21, 18, and 13, according to Genomed.

Aneufast is intended to amplify DNA extracted from fresh prenatal samples such as amniotic fluids, chorionic villus sampling, or fetal blood; but it can also be used to analyze neonatal and adult blood or tissue samples.

In the largest North American study of its type to date, published this month in *Genetics in Medicine*, clinical researchers from North York General Hospital and Mount Sinai Hospital in Toronto validated Aneufast on 200 known, blinded, prenatal DNA specimens; and then prospectively validated the kit using 1,069 amniotic fluid specimens, and compared the results with both karyotyping and FISH.

They showed that QF-PCR testing had analytical sensitivity and specificity of 98.9 percent and 100 percent, respectively, for the validation specimens; and sensitivity and specificity of 98 percent and 100 percent, respectively, in the prospective study, leading them to conclude that QF-PCR is a viable alternative to interphase FISH as a rapid prenatal diagnostic test for common aneuploidies.

The researchers also noted that QF-PCR shows promise as a replacement for full karyotyping in low-risk pregnancies in Canada, and that other studies conducted in the United Kingdom have shown that using QF-PCR as a standalone test for pregnancies without ultrasound anomalies resulted in faster turnaround times, lower costs, and less ambiguity.

"I would say the gold standard in prenatal cytogenetics is karyotyping," said Diane Allingham-Hawkins, first author on the *Genetics in Medicine* study, and currently director of Genetic Test Evaluation Services at health technology research and consulting company Hayes.

"The issue is that karyotyping is expensive, and most of the time you're looking for aneuploidies of these five chromosomes.

"We had two thoughts: One was to replace FISH, because it's expensive, the reagents are expensive, it's labor-intensive, and it's not conducive to automation," Allingham-Hawkins said. "The other thought was ... to be able to offer a rapid rule-out or diagnostic test for Down syndrome and the other

major aneuploidies — could we get this to such a point that we could offer it to every woman who had a procedure, in order to alleviate stress and all of those things that come along with having a prenatal diagnostic procedure?"

Allingham-Hawkins added that such a scenario is plausible, but did note that more research is first needed in North America to validate the test. "The problem there is that historically you've done a karyotype on all women who've had a prenatal procedure, and it feels like a step backwards to not do it, even though the vast majority are going to be normal," she said. "Will it be perceived as a change in care, where women are getting less care than [they] are used to?"

In fact, QF-PCR testing has all but replaced both FISH and karyotyping in low-risk pregnancies in Europe. The most recent Canadian study follows on the heels of a much larger study, published in 2009 in *Prenatal Diagnostics*, which detected aneuploidies involving chromosomes 21, 18, 13, X, and Y with 100 percent specificity in 43,000 clinical samples collected over nine years.

"If you read one paper, you'd be better off reading the one that looked at more than 40,000 samples," Genomed's O'Connor said. "But the new paper was written from the Canadian perspective ... and confirms that what we've been doing in Europe for the last five years or so is definitely preferable to what they were doing before in terms of speed, accuracy, and throughput."

Regardless of whether QF-PCR testing can soon become the prenatal aneuploidy test of choice in Canada or the rest of the world, it will likely be a much longer road in the US, where Aneufast or a competing test, Gen-Probe's Elucigene QST<sup>®</sup>R, have yet to receive marketing clearance from the US Food and Drug Administration. Like Aneufast, the Elucigene test is validated for use on ABI genetic analyzers.

According to O'Connor, the FDA indicated that it couldn't approve Aneufast until an ABI system that performs the requisite fragment analysis is also approved. Aneufast works on any ABI capillary electrophoresis platform, "from a one-capillary [system] right up to the 96-capillary that is very common in the US for higher throughput," O'Connor said. "

"The US is still primarily using FISH and old technology," O'Connor said. "But only in the US do they want the test linked to the particular platform by validating some of the software and some other things."

Life Tech is currently preparing to submit its 3500 Dx system, which is already CE-IVD marked in Europe, for 510(k) approval in the US, Life Tech spokesperson Patty Zamora told *PCR Insider* in an e-mail this week.

"There are significant differences in the FDA's process and requirements for 510(k) filing and registration, as compared to the CE-IVD registration process," Zamora said. "We anticipate filing our application for 510(k) registration of the 3500 Dx Series before the end of calendar year 2011, and will inform the community when we are granted FDA approval."

O'Connor said that Genomed and Molgentix have been in discussions with Life Tech about pursuing such approval and offering Aneufast on the 3500 Dx. Zamora confirmed that Life Tech has "discussed the possibility of this type of cooperative work with Molgentix, as well as other similar companies," but that it has not formalized any agreement with Molgentix.

"When they get FDA approval, we've already done all the work for FDA approval, and [our test] will appear in America," O'Connor said.

Meantime, competing firms are developing prenatal aneuploidy tests on other genomic platforms — such as comparative genomic hybridization microarrays and next-generation sequencers — that are also looking to displace traditional platforms such as karyotyping and FISH.

For example, PerkinElmer subsidiary Signature Genomics offers a prenatal testing service on a CGH array that screens for aneuploidy on 24 chromosomes as well as multiple other chromosomal disorders. The test is run in Signature's CLIA-approved lab and is intended as a complement for karyotyping. And Sequenom is developing a blood-based, noninvasive test for Down syndrome on the Illumina HiSeq 2000 that it plans to launch as a laboratory-developed test before the end of the year.

---

Have topics you'd like to see covered in *PCR Insider*? Contact the editor at [bbutkus \[at\] genomeweb \[.\] com](mailto:bbutkus@genomeweb.com).